GRA.LE.D.

GRApevine LEaf Digitalization

Version: 2.04

User Manual

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What is GRA.LE.D?

GRA.LE.D. (GRApevine Leaf Digitalization) is free raster graphic software exclusively built up for digital analysis of *Vitis* leaves based on the standard ampelographic measurements published by OIV (International Organization of Vine and Wine: 2nd Edition of the OIV descriptor list for grape varieties and *Vitis* species).

During morphological description of grapevine cultivars have discovered that leaf provides numerous data which could be statistically analysed. For facilitating objective description we created a free software which we think may help in this process.

We appreciate your feedback, any suggestions or ideas are welcome, and please feel free to send us your questions about the GRA.LE.D software.

Contact us at: laszlo.baranyai@uni-corvinus.hu; bodor.peter@uni-corvinus.hu

This program is freely distributed AS IS in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE.

Citing GRA.LE.D.

Please refer to this software in your publication as:

GRA.LE.D version 2.04, Corvinus University of Budapest, Hungary, 2014

You can download from:

- http://www.laaz.hu/en/software/graled (developer)
- http://szoleszet.uni-corvinus.hu/index.php?id=56232 (researchers)

It is highly appreciated if you cite this software or any publication announced on the software website.

How to install?

Installing GRA.LE.D. is quite easy. Download the zip compressed package and save it on your hard disc or flash drive, then extract and open GRA.LE.D. executable.

How to use GRA.LE.D.?

Sampling and digitalization: collect your leaf sample, and scan it even with an ordinary desktop scanner on **300 dpi** (recommended value). Save images using **bitmap** (BMP) or **JPEG** (JPG) format. Position your samples consequently with the upper side up or down. Such uniform set of images is easy to handle for any purpose. It is recommended to place your sample in the scanner with petiole up. Processing method is not sensitive to rotation or other adjustment variations but uniformity makes batch processing much easier.

Please note that precise measurements could be carried out with some criteria:

- leaf has to be intact without any damage
- flat the leaf as much as it is possible
- mark overlapping*

* Overlapping modify your results in two ways: give false result of the leaf area and make difficulties when you measure opening of the petiole sinus. See chapter: OVERLAPPING

Measurement:

(Before start print the supplementary figure to follow biometric points in the GRA.LE.D.

1 Start GRA.LE.D. and open a file from your hard disc or flash drive with “OPEN”.

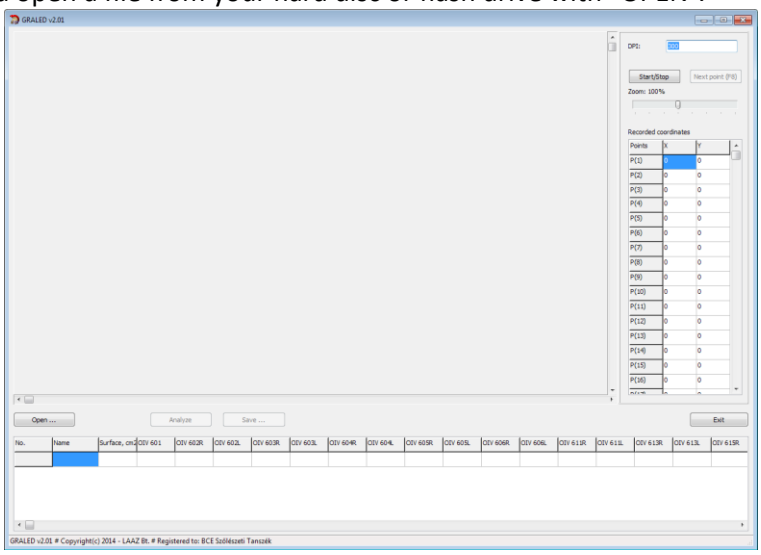


Figure 1

2 Click on “OPEN” and choose your file from your hard disc or flash drive with the selection of file type: Bitmap (*.bmp) or JPEG (*.jpg).

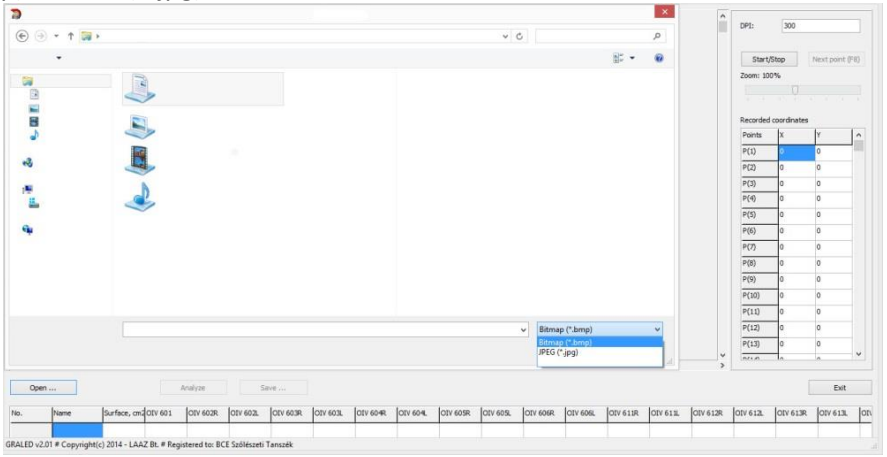


Figure 2

3. Zoom out and adjust zoom for proper marking of the points
4. Press “START/STOP” 2 times

5. Start marking the 1. biometric point with click on the 1. landmark point on your sample. Coordinates of the point appear in the right table.

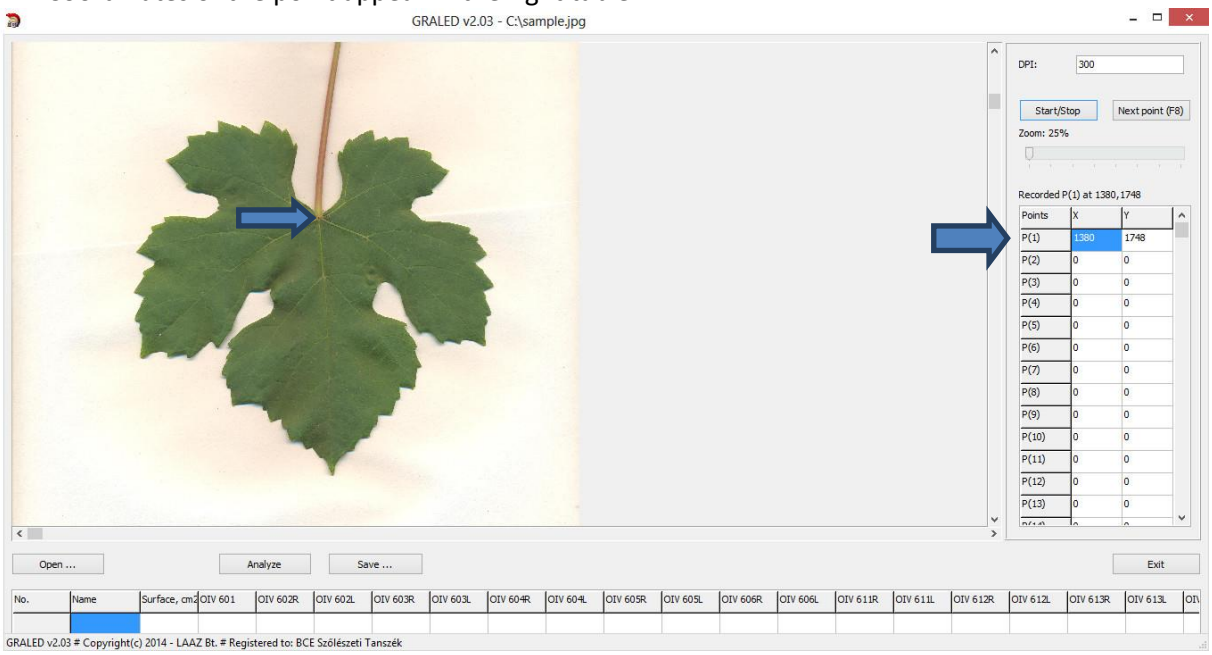


Figure 3

6. Red cross also appear on the picture so that you can check position. In case of false positioning, click again on the correct landmark point. Coordinates will be updated automatically.

7. Press F8 for next and click to 2. landmark point. On the top of the table on the left you can check which point you are currently reading.

Figure 4

8. Go on until you finish with the 32 landmark points and Table fill with coordinates. (Figure 5)

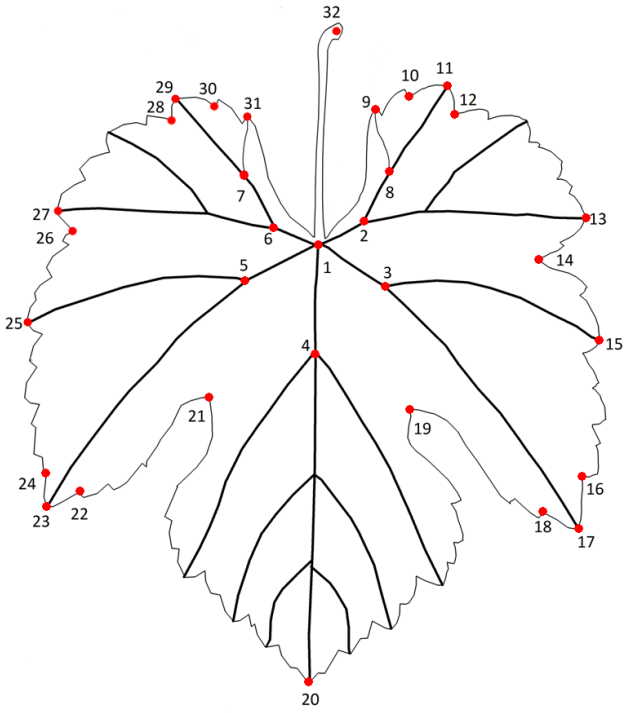


Figure 5

9. When all points are defined press “ANALYZE”

Figure 6

10. Data appear in the lower table.

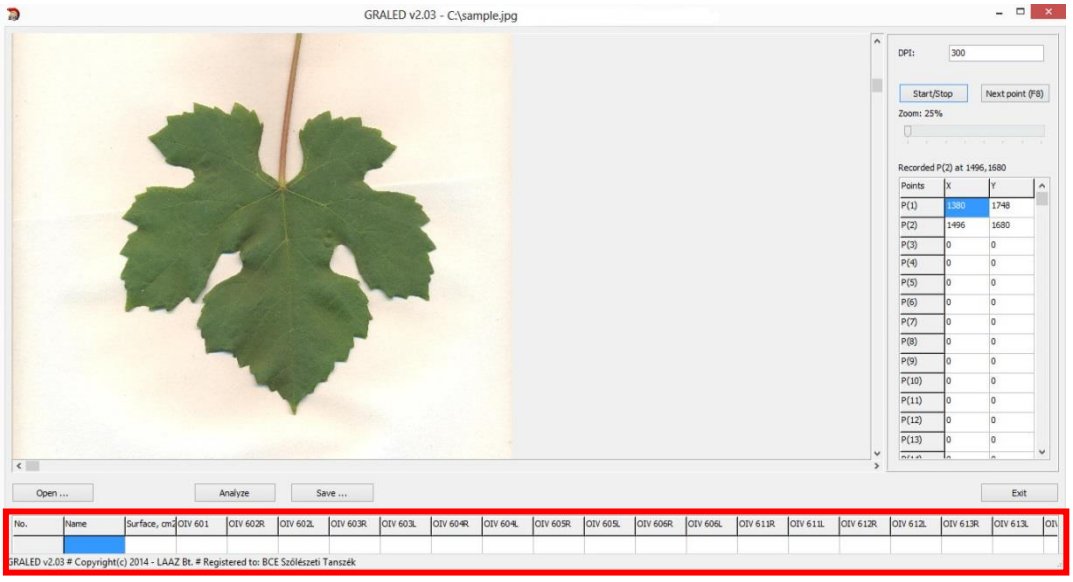


Figure 7

11. You have the possibility to measure further samples with opening a new image or stop measurements after a single sample.

12. Click “SAVE...” to export table data.

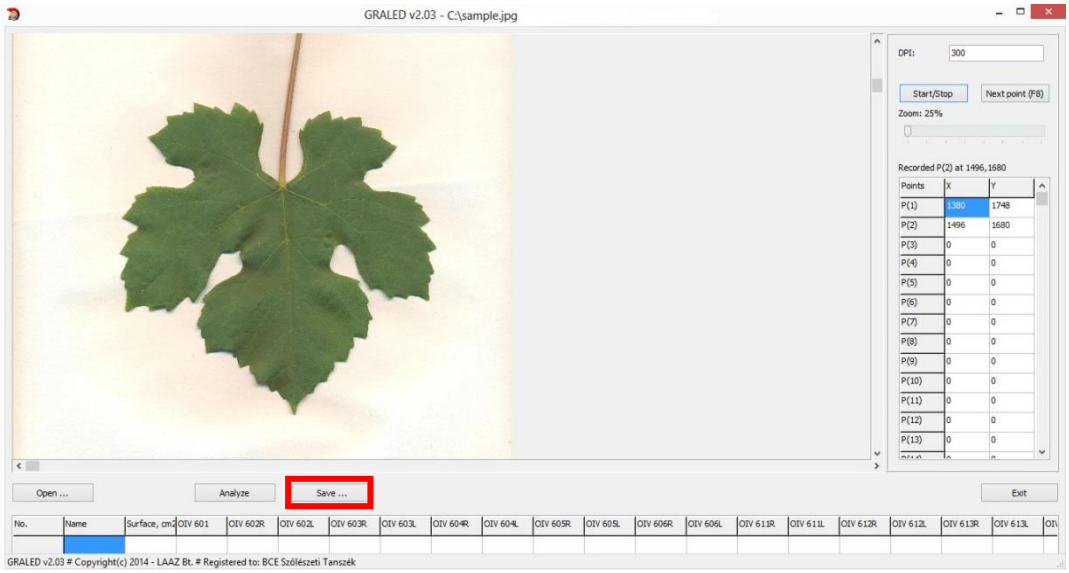


Figure 8

Landmark points

Landmark point	Leaf characteristic
1	insertion point of leaf petiole and lamina
2	first ramification of N_3
3	first ramification of N_2
4	first ramification of N_1
5	first ramification of N_2
6	first ramification of N_3
7	first ramification of N_4
8	first ramification of N_4
9	top of the teeth N_5
10	base of the teeth N_4
11	top of the teeth N_4
12	base of the teeth N_4
13	top of the teeth N_3
14	base of the sinus
15	top of the teeth of the first ramification N_2
16	base of the teeth N_2
17	top of the teeth N_2
18	base of the teeth N_2
19	base of the sinus
20	top of the teeth N_1
21	base of the sinus
22	base of the teeth N_2
23	top of the teeth N_2
24	base of the teeth N_2
25	top of the teeth of the first ramification N_2
26	base of the sinus
27	top of the teeth N_3
28	base of the teeth N_4
29	top of the teeth N_4
30	base of the teeth N_4
31	top of the teeth N_5
32	base of the petiole

Export data:

You can export your data in tab delimited plain text (TXT) file format to your hard disc. This structured data can be imported to spreadsheet applications and statistical softwares. In the text file, sample name will be the same as the picture filename was.

Troubleshooting

1. Following dialog bar appear (Figure 9)

You did not select one or more biometric point of the leaf.

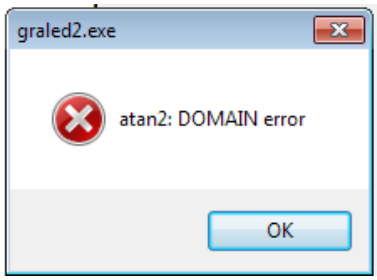


Figure 9

2. Your exported txt. file is empty

You forgot to press “ANALYZE” after the measurements.

3. During the data analysis you have outliers

Your sample set was not uniform, or you made mistake during the measurements.

4. After finish with the marking of 32 points you still have empty cell in the table

You forgot to press F8 in one or more times during the measurements and coordinates are overwritten. If you miss one point or marked a wrong position, correct the mistake by pressing F8 until you reach the point needs to be replaced.

5. You have “missing” landmark point on your sample

You should aim to collect intact samples without any damages, but it not always come true. It is usually happens that one or more parts are missing on the sample caused by hail, senescence of the leaves or even phytotechnical treatments. When you reach to missing point simply presses F8 and skip it. Later You have to handle missing data in statistical analysis. In the present example (Figure 10) landmark point 13 is missing. In this case you reach and record landmark point 12 and press F8 two-times and indicate landmark point 14. In your exported data all missing coordinates and missing OIV values will be indicated with “N.A.”

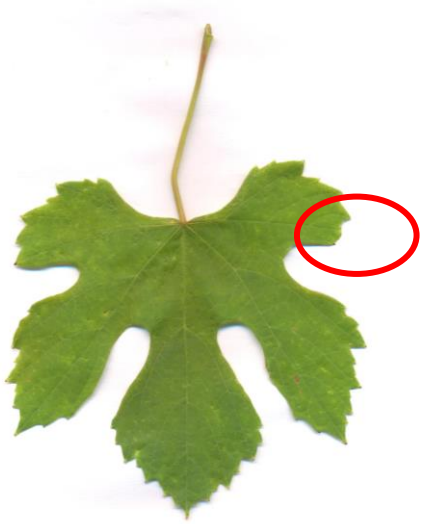
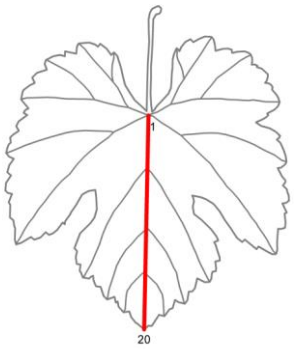
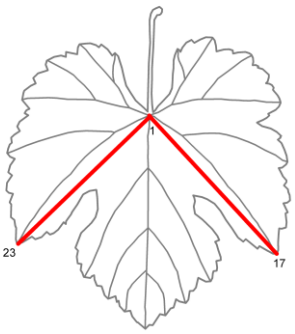
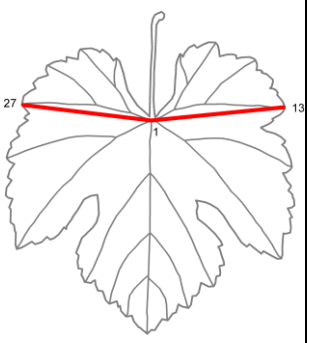
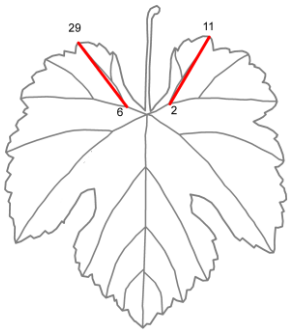
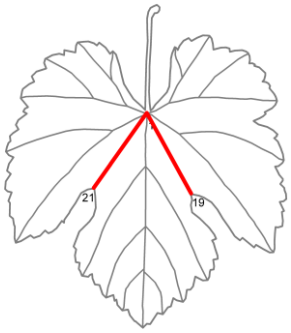
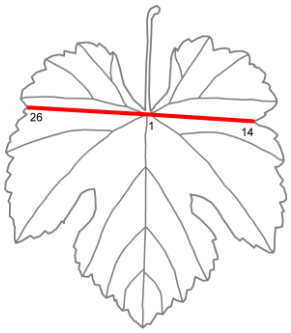
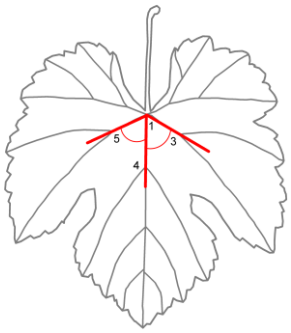
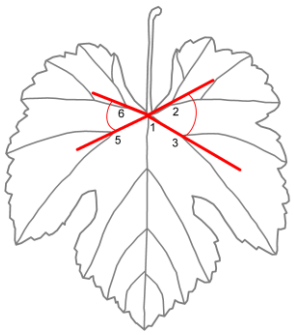
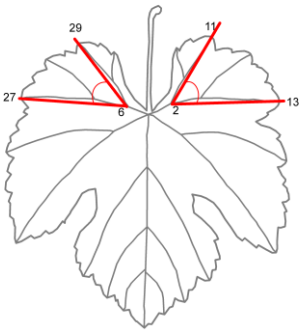


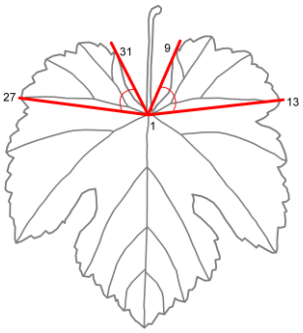
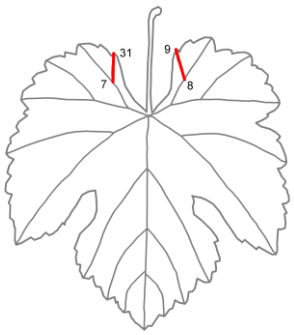
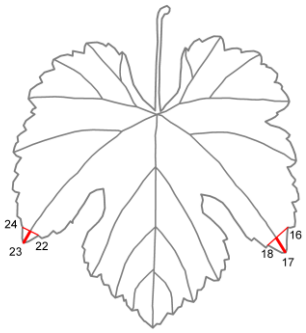
Figure 10

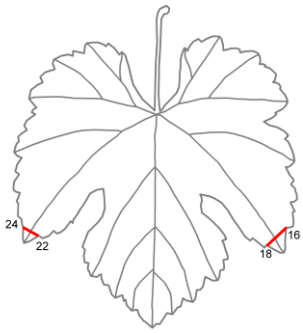
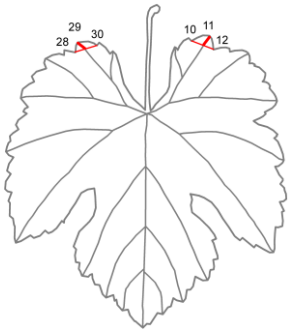
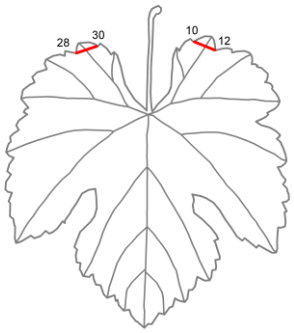
Biometric points given by the software refer to the following OIV descriptors

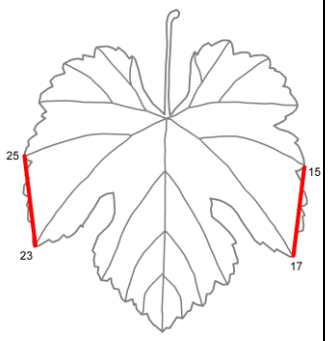
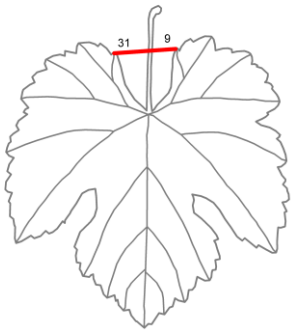
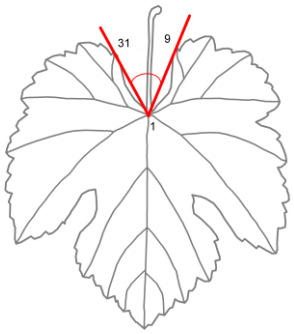
OIV code	OIV601	
Description	Mature leaf: length of vein N_1 To be measured on at least 10 mature leaves from the middle third of several shoots: length of vein N_1.	
Unit	mm	1-20
OIV code	OIV602	
Description	Mature leaf: length of vein N_2 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: length of vein N_2.	
Unit	mm	1-17; 1-23
OIV code	OIV603	
Description	Mature leaf: length of vein N_3 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: length of vein N_3.	
Unit	mm	1-13; 1-27

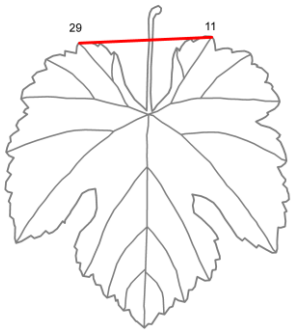
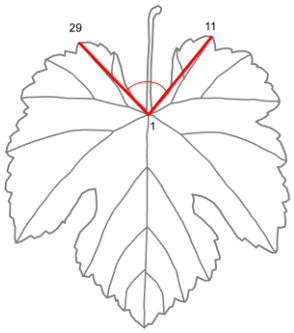
OIV code	OIV604	
Description	Mature leaf: length of vein N_4 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: length of vein N_4.	
Unit	mm	2-11; 6-29
OIV code	OIV605	
Description	Mature leaf: length petiole sinus to upper lateral leaf sinus To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: distance from petiole sinus to upper lateral leaf sinus.	
Unit	mm	1-19; 1-21
OIV code	OIV606	
Description	Mature leaf: length petiole sinus to lower lateral leaf sinus To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: distance from petiole sinus to lower lateral leaf sinus.	
Unit	mm	1-14; 1-26

OIV code	OIV607	
Description	Mature leaf: angle between N1 and N_2*) measured at the first ramification *Code Nos OIV 601 and OIV 602) To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of 10 leaves: the angle between N_1 and N_2 measured on the tangents formed before these veins first branch.	
Unit	mm	1-3 Θ 1-4; 1-5 Θ 1-4
OIV code	OIV608	
Description	Mature leaf: angle between N_2 and N_3*) measured at the first ramification *Code Nos OIV 601 and OIV 602) To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of 10 leaves: the angle between N_2 and N_3 measured on the tangents formed before these veins first branch.	
Unit	degree °	1-2 Θ 1-3; 1-5 Θ 1-6
OIV code	OIV609	
Description	Mature leaf: angle between N_3 and N_4*) measured at the first ramification *Code Nos OIV 601 and OIV 602) To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: the angle between N_3 and N_4 measured on the tangents formed before these veins first branch	
Unit	degree °	2-11 Θ 2-13; 6-27 Θ 6-29

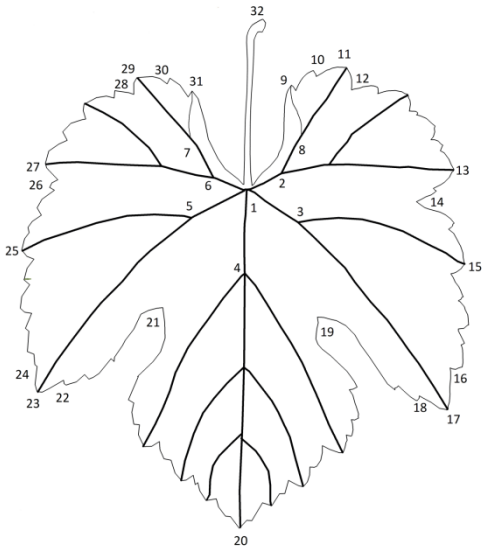
OIV code	OIV610	
Description	Mature leaf: angle between N3 and the tangent between petiole point To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: angle between N3 and the tangent between petiole point and the tooth tip of N5.	
Unit	degree °	1-9 Θ 1-13; 1-27 Θ 1-31
OIV code	OIV611	
Description	Mature leaf: length of vein N5 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: length of vein N5.	
Unit	mm	
OIV code	OIV612	
Description	Mature leaf: length of tooth N2 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: length of tooth of N2.	
Unit	mm	

OIV code	OIV613	
Description	Mature leaf: width of tooth N2 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: width of tooth of N2.	
Unit	mm	16-18; 22-24
OIV code	OIV614	
Description	Mature leaf: length of tooth N4 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: length of tooth of N4	
Unit	mm	
OIV code	OIV615	
Description	Mature leaf: width of tooth N4 To be measured on at least 10 mature leaves from the middle third of several shoots on both halves of the leaf: width of tooth of N4.	
Unit	mm	10-12; 28-30

OIV code	OIV617	
Description	Mature leaf: length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 Measurement on at least 10 mature leaves from the middle third of several shoots, on both halves of the leaf.	
Unit	mm	15-17; 23-25
OIV code	OIV618	
Description	Mature leaf: opening/overlapping of petiole sinus E: To be measured on at least 10 mature leaves from the middle third of several shoots: Wide open sinuses: measure the distance between the blades at half the height of the petiole sinus. The value will be recorded as negative. Overlapping lobes: measure at the widest overlap between the blades. The value will be recorded as positive.	
Unit	mm	9-31
OIV code	OIV618	
Description	Mature leaf: opening/overlapping of petiole sinus	
	E: To be measured on at least 10 mature leaves from the middle third of several shoots: Wide open sinuses: measure the distance between the blades at half the height of the petiole sinus. The value will be recorded as negative. Overlapping lobes: measure at the widest overlap between the blades. The value will be recorded as positive.	
Unit	degree °	1-9 Θ 1-31

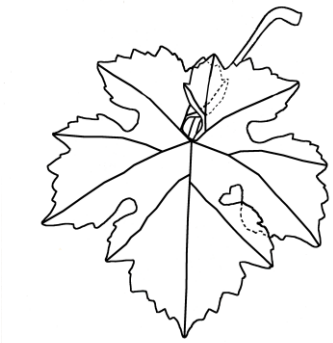
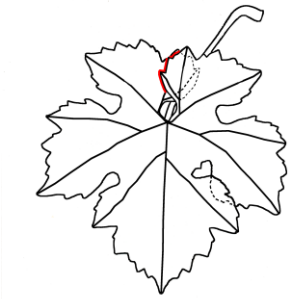
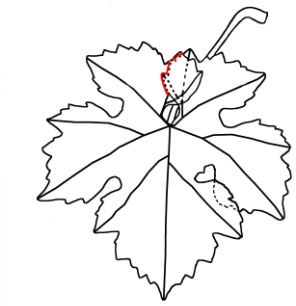
OIV code	OIV618	
Description	Mature leaf: opening/overlapping of petiole sinus	
	E: To be measured on at least 10 mature leaves from the middle third of several shoots: Wide open sinuses: measure the distance between the blades at half the height of the petiole sinus. The value will be recorded as negative. Overlapping lobes: measure at the widest overlap between the blades. The value will be recorded as positive.	
Unit	mm	11-29
OIV code	OIV618	
Description	Mature leaf: opening/overlapping of petiole sinus	
	E: To be measured on at least 10 mature leaves from the middle third of several shoots: Wide open sinuses: measure the distance between the blades at half the height of the petiole sinus. The value will be recorded as negative. Overlapping lobes: measure at the widest overlap between the blades. The value will be recorded as positive.	
Unit	degree °	1-29 Θ 1-11

NON OIV descriptors provided by the software

NON-OIV descriptors	Distances between the points	
	1-2	
	1-3	
	1-4	
	1-5	
	1-6	
	1-31	
	1-9	
	1-29	
	1-11	
	1-32	
	27-13	
	25-15	
	23-17	
	11-13	
	13-17	
	17-20	
	20-23	
	23-27	
	27-29	

Overlapping

To handle overlapping you should make a marking on the leaf before scanning as the following schedule suggest.

1.	Flat the leaf with overlapping on the petiole sinus.	
2.	Mark the overlapping with color marker.	
3.	Replace the overlapped lobes.	
4.	Scan the leaf.	

Supplementary figure